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Formulation And Evaluation Of Topical Nanogel Of Bacitracin Loaded Nanosponges

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ABSTRACT
Battling localized bacterial infections requires advanced drug delivery. This research introduces a Nanosponges-based nanogel designed for controlled bacitracin release, targeting gram-positive pathogens. Motivated by the need for enhanced therapy and reduced dosing, this novel system aims to revolutionize localized antibiotic treatment. Nanosponges, encapsulating bacitracin, were created using emulsion solvent diffusion method, optimized via 3^2 factorial design. These were incorporated into a nanogel using gelling agents. Evaluations included particle size, FTIR, in vitro release, entrapment efficiency, SEM, and XRD. Specific formulations showed promising attributes: controlled drug release, efficient entrapment, and suitable particle size. Morphological studies confirmed nanosponges formation and drug dispersion. In vitro data suggested extended release. This study establishes nanosponges nanogel as a viable platform for controlled bacitracin delivery. The sustained release offers potential for improved infection management. This research contributes valuable data to antibiotic drug delivery, paving the way for further study."

Keywords: *Nanosponges , Bacitracin, Nanogel, Encapsulation, Anti-bacterial*

1. INTRODUCTION

The biggest organ in the body, the skin makes up 15% of an adult's total weight and serves as an essential defence against the elements. It is essential for preserving general health and acts as the first line of defence. The appearance of skin, including hair and nails, conveys both social cues and indicators of overall well-being. The adult's apparent surface area is a modest 1.2 to 2.0 m^2. The body's generally smooth contours and absence of significant folds, plications, and villi are the main causes of its small surface area. Its three primary layers—the epidermis, dermis, and hypodermis—make up its intricate structure. It acts as an essential sensory contact with the external environment, controls body temperature and secretions, and offers self-healing protection against chemical and physical damages. There are two distinct layers to the skin. The epidermis is a quickly replicating surface layer that can easily restored from any harm caused by external interactions. Everybody has a protective layer of some kind that keeps out tiny molecules. They are all made to move faster than others. The skin can function as a sensory organ that can identify and assess inputs from the environment due to projections of the central nervous system.

The Bacterial infection that caused in skin

- Impetigo
- Folliculitis
- Carbuncles and furuncles
- Minor skin abscesses

MINOR SKIN ABSCESSES

The abscess arises in many tissues and organs of the body, the most important of which are subcutaneous tissue, lymph nodes, soft and adipose tissue around the anus, and breasts in pregnant or lactating women and at the root of the teeth. Most of the skin abscess tends to melt within 7–10 days, the estimate variable spread significantly. The Common cause of skin abscesses is *Staphylococcus aureus*, and *Streptococcus pyogenes* and are the most common types of bacteria that cause skin abscesses in the following areas of the body; the head and neck, parties, armpits, trunk.

CUTS, SCRAPES AND MINOR BURN

Cuts, scrapes, and minor burns are common occurrences in daily life, often resulting from accidental contact with sharp objects, rough surfaces, or heat sources. These common wounds, while often superficial, disrupt the skin's integrity, creating a potential entry point for pathogens and posing a risk of infection. Proper wound care is essential not only to prevent such infections but also to facilitate the complex process of tissue repair and regeneration. This process involves a cascade of cellular and molecular events, and its successful completion determines the speed and quality of healing.

PROGRESSION OF CUTS, SCRAPES AND MINOR BURN

The stages involved in progression of a cut, scrape, or minor burn typically follows:

1. **Bleeding:** The first stage is bleeding, which usually stops on its own within a few minutes.
2. **Cleaning:** The next stage is cleaning the wound to remove any dirt or debris. This can be done with soap and water or with an antiseptic solution.
3. **Covering:** Once the wound is clean, it should be covered with a bandage to protect it from further contamination.
4. **Healing:** The final stage is healing, which can take anywhere from a few days to a few weeks, depending on the severity of the injury.

MAIN CAUSES:

- **Cuts:** Cuts are usually caused by sharp objects, such as knives, scissors, or broken glass.
- **Scrapes:** Scrapes are usually caused by contact with rough surfaces, such as pavement or concrete.
- **Minor burns:** Minor burns are usually caused by contact with heat sources, such as hot water, stoves, or irons. They can also be caused by exposure to chemicals or electricity.
- **Minor skin abscess:** It is due to bacterial infection such as Staphylococcus aureus.

BACITRACIN:

Bacitracin was discovered in 1945 by Meleney and Johnson and is produced by cultures of bacillus, Bacillus subtilis and Bacillus licheniformis. Its potent antibiotic activity mainly acts against cocci and bacilli of Gram-positive bacteria, including Staphylococcus, Streptococcus, and Clostridium difficile, but also against Archaebacteria, such as Methanobacterium, Methanococcus and Halococcus.

Bacitracin is not administered systemically due to its nephrotoxicity, so this route of administration is used as a last resort. Its oral administration is possible, since the drug is not absorbed by the gastrointestinal system.

The main goal of antimicrobial therapy is to eradicate invading microorganisms by delivering an optimal amount of active drug to the site of infection. The ability of an antibiotic drug to reach effective concentrations at the site of infection is related to the physicochemical and pharmacological characteristics of the molecule. Polypeptide antibiotics are potent antimicrobial agents, however they are not effective against facultative intracellular pathogens during their intracellular growth phase, because penetration of antimicrobial agents through the cell membrane into the cytoplasm is insufficient.

COMMON BACITRACIN SIDE EFFECTS INCLUDE:

- Mild skin irritation like itching, rash
- Skin irritations include redness, burning or stinging at the application site.
- Eye irritations which have blurred vision.
- It is rare but serious side effect that can occur with systemic use.

BACITRACIN AS NANOSPONGES:

Nanosponges are class of materials made up of tiny sponge-like structure with narrow cavity of few nano meter, with an average diameter below 1μm These are spongy spheres that have countless interconnected empty spaces called voids. These

voids help in entrapping a wide variety of drugs which for themselves are poorly soluble and encompass them in the matrix and thus improve their bioavailability. They have an inner lipophilic cavity with hydrophilic branching on the outside, which facilitates carrying both hydrophilic and lipophilic drug molecules.

BACITRACIN AS NANOGEL:

Bacitracin nanogel is an innovative topical formulation designed to enhance the treatment of bacterial infections and promote wound healing. Bacitracin, a potent antibiotic effective against Gram-positive bacteria, is loaded into these nanosponges to ensure sustained drug release and targeted therapy. The nanogel formulation is optimized for making it suitable for managing minor cuts, burns, and bacterial infections. Additionally, its antibacterial activity has been demonstrated through in vitro studies, highlighting its potential as a promising solution for localized dermatological applications.

2. METHODOLOGY

PREFORMULATION STUDIES

CHARACTERISTICS

To examine the characteristic of the Bacitracin raw drug, 0.1 units of the drug were placed in a glass watch and observed. The drug appeared as a white to pale buff powder and was odourless.

SOLUBILITY STUDIES

It is readily soluble in water, soluble in ethanol, soluble in dichloromethane, slightly soluble in acetone, benzene, and ether, and practically insoluble in chloroform.

STANDARD CURVE FOR BACTRACIN

100 mg of Bacitracin drug was dissolved in a 50 ml vol flask with distilled water. This resulting was termed as the stock solution. Following this, 1ml of stock solution is diluted with distilled water to make up to volume of 10ml. This resulted in a new solution with a concentration of 10µg/ml. The same procedure was repeated for concentrations of 20µg/ml, 30µg/ml, 40µg/ml, 50µg/ml. Each of these solutions was referred to as standard solution. The absorbances of these standard solutions were subsequently measured using a UV-visible spectrophotometer at a wavelength of 474 nm.

FOURIER TRANSFORMEDD INFRARED (FTIR) SPECTROSCOPY

FTIR spectroscopy was employed as a method to observe the drug compatibility between Bacitracin and excipients. Also, with Bacitracin Nanosponges (BNS).

3. METHOD OF PREPARATION

ANALYSIS OF FACTORIAL DESIGN:

To create a Nanosponges' gel, an extensive review of literature and experimentation with formulations is essential. The amount, concentration, and proportion of EC and PVA used play a critical role in determining the properties of the Nanosponges. These variables are key in developing a stable and effective carrier system. A 3^2 factorial experimental design was employed to examine the physicochemical properties of the Nano sponges gel containing Bacitracin.

PREPARATION OF BACITRACIN NANOSPONGE:

To make Nanosponges we are utilizing emulsion solvent diffusion method. Bacitracin and Ethyl cellulose were mixed with Dichloromethane (organic phase). Polyvinyl alcohol was dissolved in distilled water (aqueous phase). Which were added previously made organic phase mixture. The above mixture was placed on stirrer for 2 hrs at 1000 rpm (was shown in the fig). The entire procedure was reiterated, adjusting the quantities of Ethyl cellulose and PVA as specified in table no below. Prepared Bacitracin Nanosponges are filtered and dried in hot air oven as powder.

Table no: 5 (Composition of drug and excipients)

	F1	F2	F3	F4	F5	F6	F7	F8	F9
DRUG	100	100	100	100	100	100	100	100	100
ETHYL CELLULOSE	200	200	200	400	400	400	600	600	200
PVA	400	500	600	400	500	600	400	500	400
DCM	20	20	20	20	20	20	20	20	20

DISTILLED WATER	100	100	100	100	100	100	100	100	100

NANOSPONGES INTO NANOGEL FORMULATION:

Bacitracin Nanosponges is prepared by using emulsion solvent diffusion method and dried as powder. In this, gel forming polymer i.e., Carbopol 934 soaked in water for 2 hrs and dispersed using magnetic agitation at 600 rpm with the help of magnetic stirrer to get smooth dispersion. To this

Triethanolamine(2%w/v) was added with agitation. Finally, ethanolic solution of best formulation of Nano sponges and propylene glycol was added in to the prepared base. Table no: 6 (composition of gel)

Table: 6

INGREDIENTS	BNS	Carbopol 934	Triethanolamine	Propylene glycol	Distilled water
G1	100 mg	0.2 gm	2ml	10ml	30ml

CHARACTERIZATION OF BACITRACIN NANOSPONGES:

.ZETA SIZER:

The average particle size of the Nanosponges was analysed by using Malvern Zeta sizer at 25°c.

ZETA POTENTIAL:

Zeta potential is an essential parameter that helps to determine the stability of the formulated Nanosponges by measuring the electrostatic charge on the surface of the particle. Zeta potential is analysed by using the Zeta sizer.

ENTRAPMENT EFFICIENCY:

After synthesizing Nanosponges, they are centrifuged at 8000 rpm for 10 minutes to separate them from the liquid medium; the resulting supernatant is then collected and analyzed using a UV spectrophotometer to identify any remaining released drug, which is essential for determining drug loading efficiency, purity, and stability of the nanosponges.

$$\%EE = \{\text{Drug added} - \text{free unentrapped drug} \div \text{drug added}\} \times 100$$

FTIR:

FTIR analysis shows the chemical structure and interaction that occurs within the formulation. The FTIR studies was performed by FTIR spectrometry. The FTIR analysis was performed in pure drug, formulated nanosponges, drug with ethyl cellulose, drug with PVA.

X-RAY DIFFRACTION:

In wide range of angle, both pure drug and Nanosponges powder were underwent further XRD analysis that confirms the structure in the voltage of 40kv at 25°C. Cu is employed as anode material that helps for the measurements.

SEM:

Scanning electron microscopy was used to analyze particle size and surface morphology was operated at 15kV. A concentrated aqueous suspension was spread over a slab and dried under vacuum. The sample was shadowed in a cathodic evaporator with a gold layer which improves the conductivity and visibility.while the image processing software helps to measure their diameters and calculate an average particle size.

CHARACTERIZATION OF BACITRACIN NANOSPONGES NANOGEL:

pH:

The acid pH will lead to skin irritation so to ensure that the pH was determined using specific instrument.

DRUG CONTENT:

A gel formulation containing the equivalent of 10 mg of Bacitracin was carefully weighed and dissolved in 10 ml of ethanol. The resulting solution was further diluted, and its absorbance was determined spectrophotometrically at 474 nm. The drug content was then calculated accordingly.

SPREADABILITY:

This determines how the prepared nanogel is spreaded over the surface of the skin.

Spreadability = {M × L/T}

ANTIBACTERIAL STUDY:

Petri plates containing 20 ml of nutrient agar medium were inoculated with a 24-hour culture of bacterial strains adjusted to a 0.5 OD value, in accordance with the McFarland standard (Staphylococcus aureus-902). Wells were created in the agar, and varying concentrations of sample gel and BCT (500, 250, 100, and 50 µg/ml) were introduced into them. The plates were incubated at 37°C for 24 hours. Antibacterial activity was evaluated by measuring the inhibition zone diameter around the wells. Gentamicin antibiotic served as the positive control for Staphylococcus aureus. The values were calculated using Graph Pad Prism 6.0 software (USA), employing the agar well diffusion method.

STABILITY STUDIES:

The optimized formulation was stored in a sealed, airtight container for short-term accelerated stability studies. It was maintained under controlled conditions of 4ºC±1ºC and 25ºC±2ºC with 60±5% relative humidity, following modified ICH guidelines. Over a period of three months, samples were analyzed monthly to monitor any changes in particle size and entrapment efficiency.

4. RESULTS AND DISCUSSION

Particle characterization:

The particle size range of nanosponges formulations varied from 1234**±0.73** nm to 3019±0.56 nm, indicating that all formulations fall within the nano range. BNS3 and BNS4 exhibited larger particle size, while BNS5 showed smaller particles. Subsequently, particle size increased for BNS6 formulation. However, particle size decreased in the BNS8 formulation, only to sharply increase again in the NS9 trial. As a result, formulations BNS3, BNS4, BNS6, BNS7, BNS9 were excluded from the Nanosponges due to their particle sizes falling within the micrometre range.

Table No:7 Particle dimensions of Bacitracin loaded Nanosponges.

S .NO	FORMULATIONS	PARTICLE SIZES (nm)
1	BNS1	1399±0.63
2	BNS2	1259±0.67
3	BNS3	3019±0.56
4	BNS4	2129±0.61
5	BNS5	**1234±0.73**
6	BNS6	1904±0.5
7	BNS7	1784±0.66
8	BNS8	1693±0.83
9	BNS9	1796±0.89

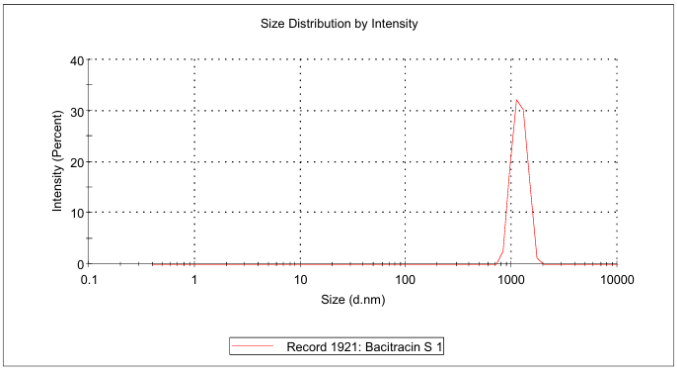

Fig no:3 Particle dimensions of Bacitracin loaded Nanosponges

ENTRAPMENT EFFICIENCY:

The nanoparticle drug delivery system's effectiveness is determined by the how much of the released near the site of action at appropriate duration. So, all the formulations were tested to calculate that how much of amount of the drug can be loaded in the Nanosponges. The BNS5 formulation demonstrates the highest drug loading capacity, achieving 88.4% in the Nanosponges. This describes BNS5 has a stronger cross-linking between the polymers and entrap more drug content.

Table No:7 entrapment efficiency.

S.NO	FORMULATIONS	ENTRAPMENT EFFICIENCY
1	BNS1	51.2±0.21
2	BNS2	55.01±0.44
3	BNS3	72.3±0.38
4	BNS4	69.6±0.24
5	BNS5	**88.4±0.15**
6	BNS6	71.2±0.43
7	BNS7	73.8±0.16
8	BNS8	60.2±0.4
9	BNS9	53.6±0.18

ZETA POTENTIAL:

The zeta potential for the optimized formulation BNS5 is –12.9 mv, this ensures good stability and prevents aggregation of the particles.

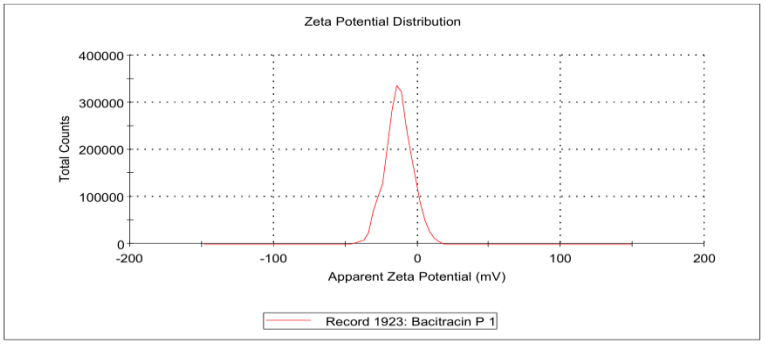

Fig no:4 zeta potential for Bacitracin loaded Nanosponges

FT-INFRARED STUDIES:

Figure (3) shows the FTIR spectra of both Bacitracin drug and Bacitracin loaded Nanosponges(BNS5) by using FTIR spectroscopy. FTIR plays a crucial role in understanding the drug-drug induced sample, drug-excipients interaction to improve the formulations. The FTIR analysis of Bacitracin and its experimental product was carried out at SRMCOP. The findings showed no indication of the drug and excipients.

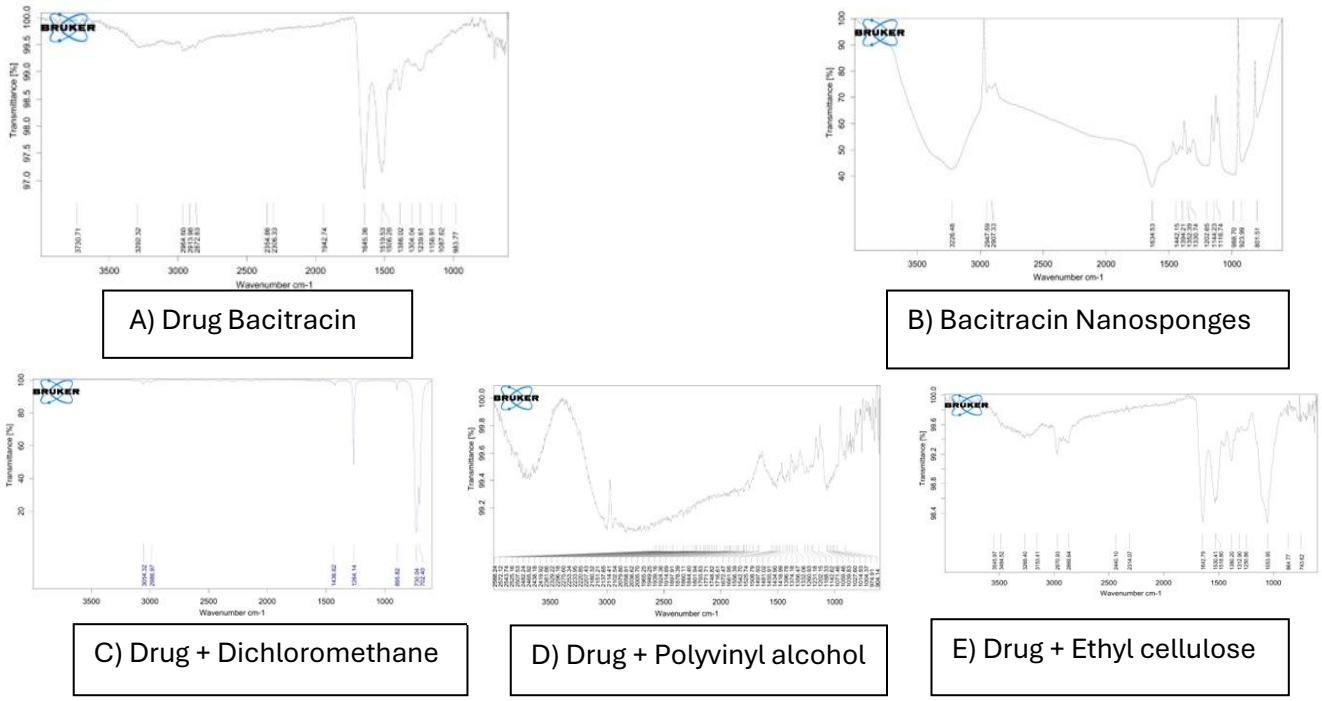

Fig no:5 FT-Infrared spectra for a) Drug Bacitracin, b) Bacitracin Nanosponges, c) Drug + dichloromethane, d) Drug + polyvinyl alcohol, e) Drug + ethyl cellulose

Table no:8 The peak wavenumber of compounds and their functional groups

Wavenumber (cm^{-1})	**Functional Group**	**Intensity (Nanosponge)**	**Intensity (Bacitracin)**	**Observation**
~3226	O-H/N-H	Strong	Strong	B Both samples show strong peaks, indicating retained hydroxyl/amine groups.
~2947–2907	C-H	Medium	Medium	Peaks are consistent, suggesting no alteration in aliphatic chains.
~1634	C=O	Strong	Strong	No significant shift, indicating carbonyl groups remain unchanged.
~1442	C-H	Medium	Medium	Identical peaks, confirming stability of alkyl group vibrations.
~1116–988	C-O	Medium	Medium	Overlapping peaks, showing no evidence of new bond formation.

The FTIR analysis reveals that the functional groups present in the Nanosponges formulation and Bacitracin sample align closely in terms of wavenumbers and intensities. There are no new peaks or significant shifts, which confirms that no chemical reaction has occurred between the components. This suggests a physical interaction, such as encapsulation, rather than a chemical modification.

Preparation of calibration curve:

The calibration curve for pure drug of Bacitracin was analysed.

Table no:9 Calibration curve for Bacitracin

Concentration(µg/ml)	Absorbance (474nm)
10	0.094
20	0.159
30	0.239
40	0.294
50	0.369

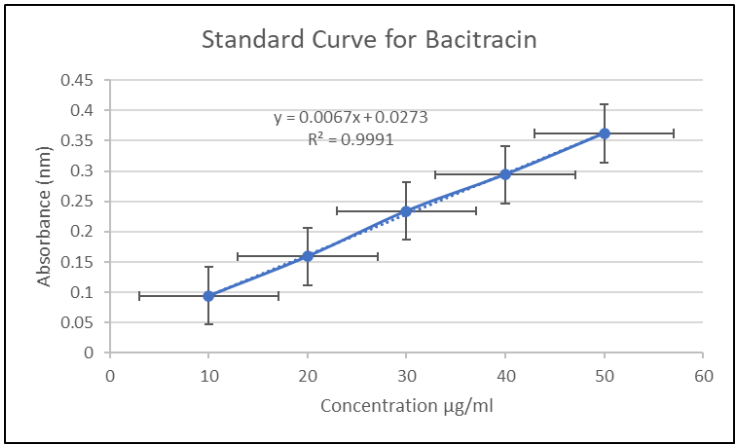

Fig no:6 Calibration curve of Drug Bacitracin

X-RAY DIFFRACTION:

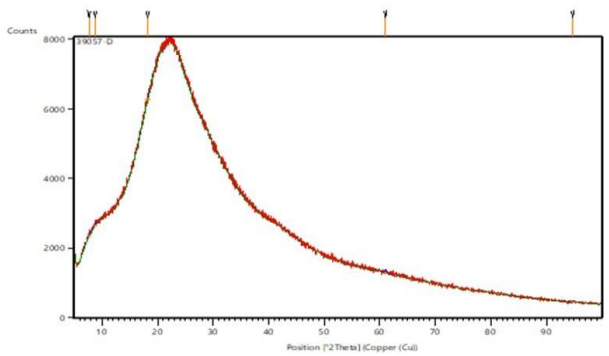

Fig no:7 a) Drug Bacitracin

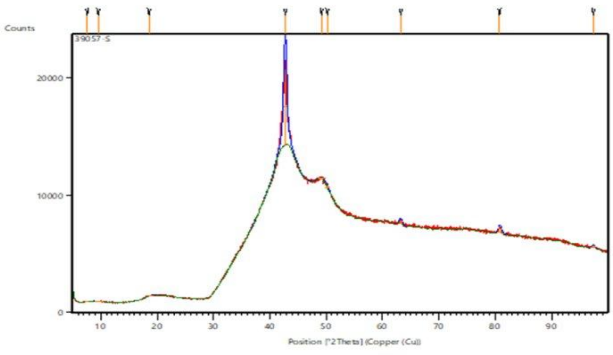

Fig no:7 b) Bacitracin Nanosponges

Table no:10 X-ray diffraction of Drug Bacitracin and Nanosponges.

Sample	Peak Positions(°2θ)	Peak Intensities	Crystalline/Amorphous Nature
Drug (Bacitracin)- 39057-D	7.65, 8.77, 18.22, 61.01, 94.73	Sharp and intense peaks	**Crystalline**
Nanosponges-39057-S	7.60, 9.57, 18.58, 42.71, 49.16, 50.20, 63.23, 80.74, 97.36	Broader and less intense peaks, except at 42.71°	**Amorphous**

The XRD results confirm that the drug is in a crystalline state before formulation, but upon encapsulation in the Nanosponges, it becomes amorphous. This suggests successful drug loading and a potential improvement in solubility, which is beneficial for drug delivery applications.

IN-VITRO DRUG RELEASE STUDY:

The in-vitro drug release study for Bacitracin Nanosponges formulations was conducted over a period of 6 hours. The BNS5(61.2%) formulation shows greater % drug release when compared to the BNS7(59.7%). This difference may be due to variations in the concentrations of EC and PVA used in the formulations. The values of % drug release versus time(hrs) are shown in the graph.

Table no:11

Time (hours)	0	1	2	3	4	5	6
BNS1	0	20.3	23.1	26.3	28.4	34.1	39.2
BNS2	0	21.2	32	37.1	39.1	46.6	48.3
BNS3	0	20.4	21.3	32.1	38.9	39.7	41.4
BNS4	0	15.8	21.2	23.2	32	38.2	46.3
BNS5	0	28.2	31.3	41.1	49.8	58.9	61.2
BNS6	0	19.6	22.5	32.1	41.3	48.1	52.2
BNS7	0	21.7	25.2	35.5	45.5	52.3	59.7
BNS8	0	19.3	24	32.5	48.2	54.3	58.2
BNS9	0	10.2	20.2	28.2	37.2	45.5	49.6

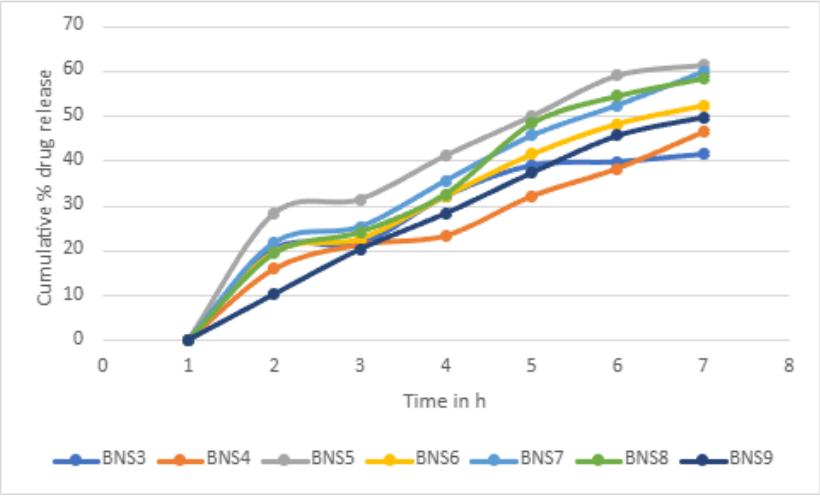

Fig no:8 % Drug release BNS

Scanning Electron Microscopy:

SEM examination of nanosponges showed spherical, porous, and nanostructured particles. The porous and sponge-like properties of the Nanosponges are clearly depicted in the figure. The soft spongy and permeable nature of the NS was demonstrated by the SEM images, which may have been caused by dichloromethane (DCM) diffusing inward into the ethyl cellulose (EC) polymeric surface of the nanosponges during creation.

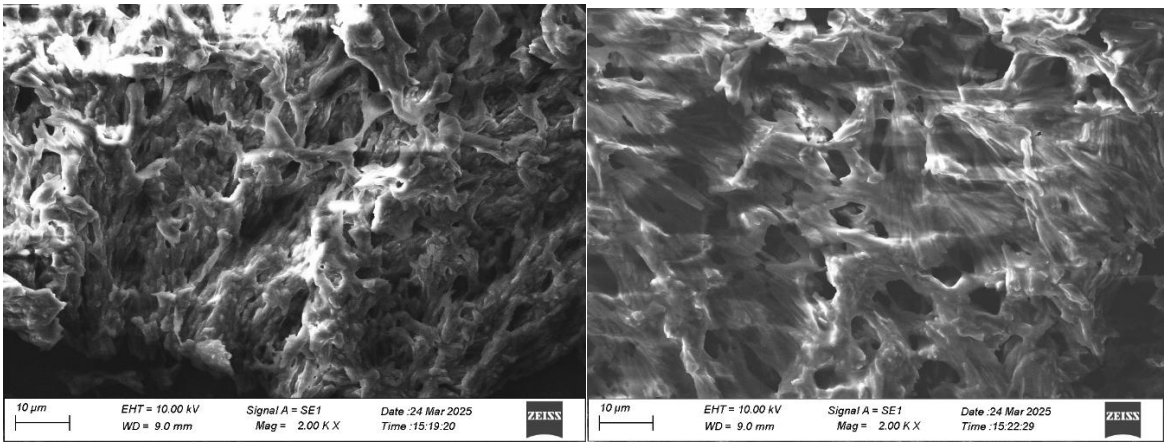

Fig 8 SEM images for BNS5

Evaluation of Bacitracin Nanosponges loaded nanogel:

pH, Spreadability and Drug Content determination:

According to rheology studies, the produced topical gel showed pseudoplastic flow as a result of shear thinning. The drug content results showed that Bacitracin was uniformly dispersed throughout the Nanosponges-based gel, and the BNS5-based optimized nanogel was easily applied to the affected skin area based on the spread ability value.

Table no:12 pH, spreadability, drug content.

S NO	PARAMETER	OPTIMIZED FORMULATION
1	pH	5.72
2	Appearance	Good
3	Odour	Good
4	Spreadability	6.5 cm - Good
5	Drug content	84.27%

***In-vitro* Release Kinetics of optimized formulation:**

The n value for the BNS in the Korsmeyer Peppas model was 0.99, which is consistent with anomalous or Non-Fickian diffusion.

Table no:13 invitro release kinetics of optimized formulation

KINETIC MODELS	HIGUCHI	KORES MEYER PEPPAS	ZERO ORDER	FIRST ORDER	HIXON CROWELL
Formulation	r^2	r^2	r^2	r^2	r^2
Optimized formulation	0.9899	0.9968 n = 0.878	0.9936	0.9560	0.9631

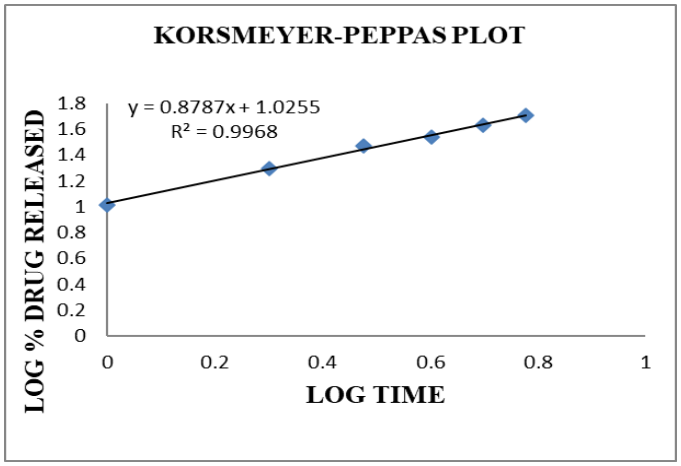

Fig no:9 invitro release kinetics of optimized formulation

Anti-bacterial activity:

Both the Gel and BCT samples exhibited concentration-dependent antibacterial activity against *Staphylococcus aureus*. Significant zones of inhibition were observed at 500 µg/ml and 250 µg/ml, indicating effectiveness at higher concentrations. However, no inhibition was observed at 100 µg/ml and 50 µg/ml, suggesting a loss of efficacy at lower concentrations. Compared to the positive control, both samples showed lower antibacterial activity. These results suggest that while the Gel and BCT formulations possess antibacterial properties, their potency is concentration-dependent and less than that of the standard antibiotic.

Table no:14 Means ± SD of zone of inhibition obtained by sample Gel and BCT against, *Staphylococcus aureus.*

S. No	Name of the test organism	Name of the test sample	Zone of inhibition (mm) Mean±SD				
			500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC
1.	*Staphylococcus aureus*	Gel	22.1±0.28	19.45±0.63	0	0	25.95±1.34
2.	*Staphylococcus aureus*	BCT	21.2±0.70	18.3±0.56	0	0	26.4±0.70

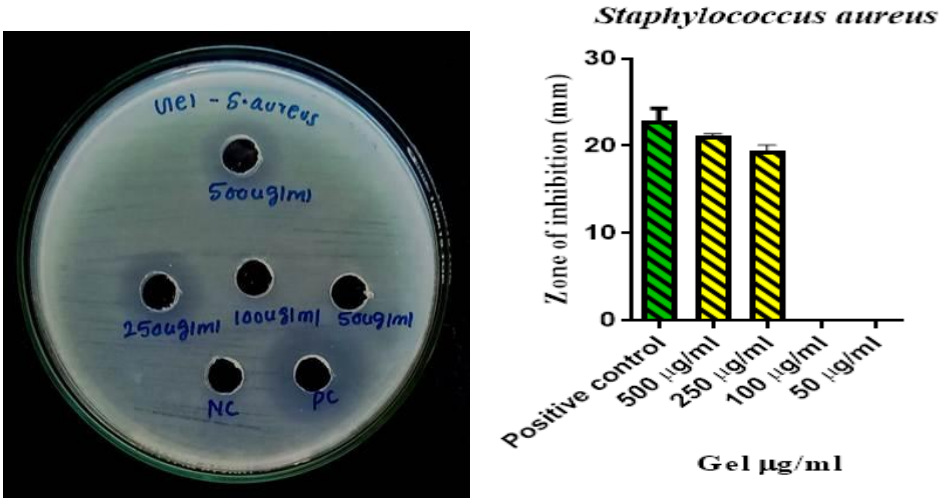

Fig no:10 Effect of sample Gel against *Staphylococcus aureus*

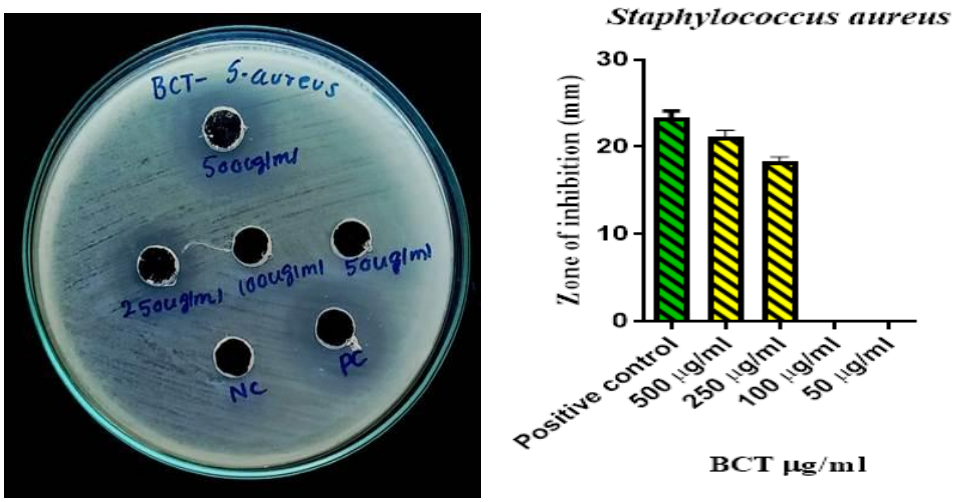

Fig no:11 Effect of sample BCT against *Staphylococcus aureus.*

8.9.6 OPTIMIZATION OF BACITRACIN NANOSPONGES:

In this work, a numerical optimization method within the Design-Expert software was utilized to simultaneously optimize both replies utilizing a desirability function. In order to find the ideal desirability value, a comprehensive grid search was carried out throughout the domain utilizing the program known as design expert.

Formulation	Predicted values			Observed values		
	Particle Size (nm)	**Entrapment Efficiency (%)**	**% Drug Release at 6th hr**	**Particle size (nm)**	**Entrapment Efficiency**	**% Drug release at 6th hr**
Optimized Formulation	1234±0.63	88.01±0.44	61.3±0.11	1239±0.45	87.3±0.19	63.1±0.39

8.9.7 STABILITY STUDY:

Optimized Nanosponges of Bacitracin was selected and placed in vials and sealed with aluminium foils. Optimized Nanosponges were stored at **4ºC±1ºC** and **25ºC±2ºC / 60±5% RH** and examined for change in particle size and entrapment efficiency for every 1 month for a period of 3 months.

Table no: 18 Stability study of Bacitracin Nanosponges

5. CONCLUSION:

The nanogel formulation incorporating Bacitracin Nanosponges (BNS) demonstrates a promising approach for sustained drug release, enhancing the potential for effective topical antibacterial therapy. BNS5, the optimized formulation, exhibited particle sizes within the nanoscale range, confirming successful Nanosponges synthesis. The formulation achieved maximum drug entrapment, ensuring efficient drug loading, and delivered maximum drug release at the 6th hour, indicating a controlled and prolonged release profile. Comprehensive nanogel evaluation, including pH, spreadability, drug content, and antibacterial activity, confirmed the formulation's suitability for topical application. The antibacterial study revealed significant efficacy against *Staphylococcus aureus*, highlighting the therapeutic potential of the nanogel. The successful incorporation of Bacitracin Nanosponges into a nanogel matrix, specifically BNS5, underscores its potential as an efficient carrier for sustained drug delivery, offering a viable strategy for improved antibacterial treatment. These findings suggest that the Bacitracin Nanosponges-loaded nanogel holds significant promise for achieving enhanced and sustained antibacterial efficacy in topical applications.

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